



# *Advances in* CLINICAL CHEMISTRY

Edited by

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VOLUME 7 • 1964



ACADEMIC PRESS  
NEW YORK AND LONDON

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ACADEMIC PRESS INC.

111 Fifth Avenue, New York, New York 10003

*United Kingdom Edition published by*  
ACADEMIC PRESS INC. (LONDON) LTD.  
Berkeley Square House, London W.1

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 58-12341

PRINTED IN THE UNITED STATES OF AMERICA

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## FOREWORD TO THE SERIES

A historian of science in years to come may well be astonished at the explosive burst of scientific activity round about the middle of the twentieth century of our era. He will be puzzled by the interrelationship between the growth of population and the rise of the standard of living; he will be interested in the increased percentage of scientists among the population, their greater specialization and the resulting fragmentation of science; he will analyze the economic and the psychological motivation of scientists; he will compare the progress of knowledge with the broadness of the current of scientific publication.

Living as we do in the midst of these events, we are hardly aware of their relatively rapid rate. What we notice is a doubling of the scientific output every ten years, regardless of contemporary political events. It is this climate which has engendered the appearance of series of reviews in dozens of disciplines. It may be with yearning or with a feeling of superiority, that we look back at such annual compendia as "Maly's Jahresberichte der Thierchemie" of one hundred years ago, which encompassed the annual progress in the zoological half of biochemistry within 300 to 400 pages.

Nowadays, that number of pages would not suffice to record the complete annual increment of knowledge in a single specialized division of the subject such as Clinical Chemistry. Media already existing furnish a comprehensive list of publications and an encyclopedic summarization of their contents; the present series of "Advances in Clinical Chemistry"—like other "Advances" series—attempts something different. Its aim is to provide a readable account of selected important developments, of their roots in the allied fundamental disciplines, and of their impact upon the progress of medical science. The articles will be written by experts who are actually working in the field which they describe; they will be objectively critical discussions and not mere annotated bibliographies; and the presentation of the subjects will be unbiased as the utterances of scientists are expected to be—*sine ira et studio*.

The bibliography appended to each chapter will not only serve to document the author's statements, it will lead the reader to those original publications in which techniques are described in full detail or in which viewpoints and opinions are expressed at greater length than is possible in the text.

The selection of the subjects in the present and in future volumes will

include discussion of methods and of their rationale, critical and comparative evaluation of techniques, automation in Clinical Chemistry, and microanalytical procedures; the contents will comprise those borderline subjects, such as blood coagulation or complement chemistry, which are becoming more chemical with increasing knowledge of the underlying reactions; in some instances the discussion of a subject will center around a metabolic mechanism or even around a disease entity.

While recognizing that the elaboration and testing of methods is of the greatest importance in a subject, part of whose function is to provide reliable, accurate diagnostic and prognostic procedures, the new series will take cognizance of the fact that Clinical Chemistry plays an essential part in the progress of medical science in general by assisting in elucidating the fundamental biochemical abnormalities which underlie disease. The Editors hope that this program will stimulate the thinking of Clinical Chemists and of workers in related fields.

HARRY SOBOTKA

C. P. STEWART

## PREFACE TO VOLUME 7

Like its predecessor, this volume of *Advances in Clinical Chemistry* ranges over the whole gamut of the subject as we defined it in our preface to Volume 6. The broad scope presented here is a deliberate act of policy—we wish to emphasize the important role clinical chemistry plays in the progress of medical science and to dispel the view occasionally held that clinical chemistry merely supplies and uses diagnostic tools for the behoof of others who alone can interpret the information supplied by those tools.

Methodology is served particularly in this volume by the review on Absorption Spectrophotometry and by that on Fractionation of Macromolecular Components of Gastric Juice—the alpha and omega of the book. In between are reviews which, while not neglecting the methodological aspects of their subjects, range more widely over the fields of dynamic biochemistry and clinical medicine. But throughout the emphasis is on clinical chemistry, the application of chemistry (or any branch of it) to the study and investigation of disease.

Once again it is a pleasure to thank our contributors and publisher for their splendid cooperation and equally to thank the many readers whose response to previous volumes has encouraged the production of this one, which we dare to hope will be as well received as were its predecessors.

*October 1964*

HARRY SOBOTKA  
C. P. STEWART

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# PRINCIPLES AND APPLICATIONS OF ATOMIC ABSORPTION SPECTROSCOPY

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## I. Introduction

One decade ago the term atomic absorption spectroscopy was familiar to only a small group of scientists, to a few chemists working on special problems, and to astrophysicists. Within the last few years, however, this analytical technique has found enthusiastic acceptance by science and industry. Several hundred papers have been published in a short time on basic research, instrumentation, development of methods, and practical application of atomic absorption spectroscopy. Several good reviews of the subject stressing various aspects have appeared (A13, D6, E2, G6, L3, L8, M6, M7, R2, R5, W15), and it seems hardly necessary to add to their number. The problems of medical laboratories and clinical

chemists are, however, unique in many respects and it was for this reason that the present article was written.

When new analytical tools become available, more often than not considerations of responsibility to the patient, practicality, and economy will keep the clinical chemist from accepting such newly developed techniques without careful deliberation. It appears that presently atomic absorption spectroscopy is slowly finding entrance into medical research and service laboratories, and there is reason to expect that this technique will find wider use and greater application than emission flame spectroscopy. Virtually all metals, with very few exceptions, can be determined by atomic absorption spectroscopy. It is anticipated that this technique not only will replace currently used analytical methods for metals, but will also make feasible the routine determination of elements now impractical by conventional means. Furthermore, the operational stability of available instruments and the simplicity of actual performance of measurements make this technique well suited for automation, by addition of an automatic sample feed and automatic recording.

Since atomic absorption spectroscopy is based on an atomic property unique for each element, the possibility exists to obtain "true" values, a challenge not to be overlooked by workers attempting to develop atomic absorption methods for a particular metal or material. Some of the original theoretical expectations in regard to freedom of interferences have not been fully substantiated in practice and almost every element has been found to suffer from one type of interference or another.

It is the object of the writer to present in this review the theoretical and instrumental basis of this analytical technique, discuss the practical methods already developed and in use, and consider some of the potentialities for the clinical laboratory.

## 2. Historical

The first to record a phenomenon caused by atomic absorption was Wollaston in 1802 in England, who observed dark bands in the sun's spectrum, although he was unaware of their cause. The same observation was made in 1814 by Fraunhofer, who, with improved instrumentation, was able to map more than 500 absorption lines in the solar radiation. Not until the days of Kirchhoff and Bunsen, however, was the true nature of the Fraunhofer lines recognized. In a series of papers Kirchhoff and Bunsen laid the foundation for modern spectrochemical analysis (K5-K10).

A typical atomic absorption experiment in the laboratory was carried out by Foucault in 1849 in Paris, as related by Stokes (S9). Foucault

passed a ray of sunlight through a carbon arc and then through a prism to produce a spectrum. In the spectrum he found the expected dark lines, but they were more completely blacked out than in sunlight without traversing the carbon arc. The mysterious power of the carbon arc to enhance these spectral characteristics of sunlight was simply due, of course, to sodium atoms in the arc deriving from sodium contamination of the carbon electrodes, causing absorption on the resonance lines of sodium, the well-known D lines in the sun's radiation.

Following the work of Lundegardh in the twenties, emission flame spectroscopy became established as an analytical tool in almost every branch of science. Although hollow cathode tubes were first studied by Paschen (P2) in 1916, and although atomic absorption spectroscopy had found occasional application, notably in the mercury vapor detector (W20), it remained for Walsh (W2) in Australia in 1955 to recognize the essential advantages inherent in absorption over emission methods and revive general interest in this technique. Shortly thereafter but apparently independently, Alkemade and Milatz (A2, A3) in Holland devised instruments and applied atomic absorption spectroscopy in their laboratory. Walsh and his co-workers have since contributed a remarkable volume of work on instrumentation and application, and patents are held by Walsh on his method in Australia, Europe, and America.

It may be mentioned here that this decade probably will see the soft lunar landing of an atomic absorption spectroscope (M8) intended to analyze surface material of the moon and relay signals back to earth. The system makes use of a solar furnace for sample vaporization and depends on the sun's spectrum as a light source.

### 3. Theoretical

Atomic absorption can simply be defined as the absorption of light by activated atoms. Such absorption occurs on very narrow spectral lines, the so-called absorption or resonance lines; their theoretical spectral width being of the order of  $0.001 \text{ \AA}$ . The lines are entirely characteristic and specific for each element and to date no two elements have been found to possess an identical resonance line. If monochromatic light of a specific wavelength is provided, it will be absorbed only by atoms of that element whose resonance line is identical with the wavelength of the light source and not by any others. A field of atoms is "opaque" for monochromatic light when resonance line and source wavelength match, but for other wavelengths it is translucent. The degree of opacity is proportional to the total number of absorbing atoms. It follows then that with a beam of specific monochromatic light the concentration of an element can be determined in a mixture of atomic species.

Atomic absorption will take place only in a field of free, neutral, activated atoms. Atomic absorption cannot be brought about by ions, by atoms bound in compounds, or by a molecular gas. When metals are heated to their boiling point, they vaporize as free atoms, provided that interaction with other elements is prevented, and it is for this reason that atomic absorption spectroscopy in its present form has found its most extensive applications in the analysis of the metallic elements.

### 3.1. ATOMIC ABSORPTION AND BEER'S LAW

As stated above the degree of atomic absorption depends on the number of absorbing atoms. If the absorbing field is spatially defined, absorption is proportional to concentration of the absorbing species. But this, in essence, is also the content of Beer's Law. The latter holds true under certain ideal conditions (L11):

- (1) The analyzing light should be perfectly monochromatic.
- (2) The light beam should be strictly parallel.
- (3) The light beam should travel in an optically homogeneous medium that does not scatter radiation.
- (4) The absorbing units (molecules or atoms) should not be close enough to one another or to other molecules that their structure or their energy levels are affected.

When these points are critically examined, it appears that these conditions theoretically are ideally realized in atomic absorption. First, the light sources used (hollow cathode lamps, discharge lamps) produce extremely sharp lines of 0.001–0.01-Å width, unobtainable by filters or monochromators. Second, parallel light beams can be obtained in most instruments of conventional dimensions and the problem of chromatic aberrations in the optical system is not encountered when working with monochromatic light. Third, under most experimental conditions, flame matrices are transparent to the analyzing light. Only where nonvaporized or unburned particles occur in the flame can light scatter be observed. Fourth, the absorbing units in a gaseous phase are considerably farther apart from one another and from other atoms and molecules than in a liquid phase, as in conventional molecular photometry.

It has been found, however, in practice that a perfectly straight analytical working curve ( $-\log T$  plotted against concentration) is seldom obtained in atomic absorption spectroscopy. The reasons for this are usually a combination of instrumental problems; broadening of the emission line of the light source due to self-reversal, Doppler and pressure broadening of the absorption lines of the atoms in the flame, failure to exclude flame emission entirely, use of a focused instead of a parallel

light beam, flame inhomogeneities, and possibly atomic fluorescence (Section 3.2) contribute to interfere with the validity of Beer's Law. The problem is discussed in detail under methodology (Section 5).

### 3.2. ATOMIC ACTIVATION AND EXCITATION

While mercury evaporates significantly at room temperatures, other metals require a supply of some form of energy to produce an atomic vapor. Most of the activated atoms in such a vapor are present in the ground state. If the supply of energy is sufficient, a small percentage of these atoms can be excited to a higher energy level, at which they remain for a very small period of time and then return to the ground state, thereby giving off their excess energy as light. Several discrete levels of excitation are possible which are reached in a stepwise quantized process. When a certain maximum level of excitation is attained, further excitation gain results in loss of the outer electron and the atom becomes an ion.

The return from higher energy states to the ground state of atoms takes place in the same stepwise transition between energy levels, resulting in emission of radiation of specific wavelengths, the characteristic emission spectrum. Atoms in the ground state can absorb on any of the wavelengths corresponding to transitions between energy levels, but it is usually the first resonance line representing the transition from the ground state to the lowest excited state where absorption is strongest. Exceptions to this are elements (iron, manganese) with complicated emission spectra, where more than one strong resonance line is encountered.

The number of excited atoms in a flame is exponentially dependent on flame temperature (Boltzmann relation). For example, for sodium, one of the most easily excited species, the ratio of excited to unexcited atoms changes at temperatures of 2000 and 4000°C from the order of  $10^{-6}$  to  $10^{-3}$ , respectively. In regard to the population of excited atoms this means an approximate 1000-fold increase, but only an insignificant decrease (less than 0.1%) of atoms in the ground state. The dependence of flame emission on flame temperature is well known (D10, P5, R6), while absorption is much less affected. Other metals, which require much higher levels of energy for excitation, produce such small numbers of excited atoms at ordinary flame temperatures, that their measurement by emission flame photometry is difficult or impossible. Most of these elements, however, at the same temperatures produce significant concentrations of atoms in the ground state which are then amenable to measurement by atomic absorption.



Most of the literature on atomic absorption neglects to mention that the light that is absorbed on atomic resonance lines causes the absorbing atoms themselves to undergo transitions to a higher energy state. Their deactivation in returning to the lower energy states by emitting light on the specific atomic lines is a form of atomic fluorescence, since excitation is from radiational, rather than thermal, energy sources. The use of atomic fluorescence flame spectrometry in the analysis of metals has recently been described (W16, W17) although the principle has been known for many years. One particular advantage of this method should be emphasized, that sensitivity can be altered by changes in the intensity of the exciting radiation.

### 3.3. FLAMES

Coincidentally with the revival of atomic absorption methods, renewed interest in flame processes has arisen among many groups of workers (B1, F7, G3, M5). Absorption techniques including the line reversal apparatus have contributed significantly to our understanding of atomic activation, excitation, atomic population densities, and temperature gradients. At the present time, the flame represents the most convenient means to create an atomic vapor under reproducible conditions. For these reasons, a short discussion of processes taking place in the flame is needed. Other means of production of activated atoms will be mentioned under instrumentation and techniques (Section 4.5).

Combustion of a fuel-air or -oxygen mixture progresses with a certain speed, the burning velocity. If the combustible mixture flows against the direction of combustion, and flow velocity and burning velocity equal each other, a stationary flame can be obtained, for instance, at the opening of a tube. When the flow velocity is greater the flame will lift off the tube opening, and when the burning velocity exceeds the former the flame will progress into the tube and cause a back-flash.

Stationary flames can be produced by premixing the fuel and air before they emerge from the burner opening; these are so-called premixed flames. If the fuel and air are fed separately and mixing occurs largely by diffusion of the two gases into each other at the burner opening, such a flame is called diffusion flame.

Stationary flames are composed of different zones: a base cone of nonluminous, unburned gases, corresponding to the transport zone in which preheating up to about 350°C takes place. This is immediately followed by a thin, highly luminous zone, the primary reaction zone, with temperatures between 1500 and 2000°C, depending on the type of